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Tyzzer disease in 19 preweaned orphaned kittens

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Abstract. *Clostridium piliforme*, the agent of Tyzzer disease, has traditionally not been considered a major pathogen of cats. We queried the database of the Pathology Service of the Veterinary Medical Teaching Hospital, University of California–Davis, for kittens <6-mo-old autopsied between 2000–2021 that had colitis, hepatitis, and/or myocarditis; 37 cases met the search criteria. Sections of colon, liver, and heart from these 37 cats were stained with modified Steiner; 19 of 37 (51%) cases had intraepithelial, Steiner-positive rods compatible with *C. piliforme* in at least one organ, confirming Tyzzer disease. The affected age range was 7–42 d (median: 17.5 d). Eighteen were orphaned kittens. Colitis was the major lesion (18 of 19) followed by random hepatitis (11 of 19). Perianal dermatitis with intraepithelial stacked rods was seen in 2 of 19. Myocarditis was not evident in any of the cases. A PCR assay for *C. piliforme* on 10 selected cases using formalin-fixed, paraffin-embedded (FFPE) blocks was positive or suspected in colon (5 of 10), liver (5 of 10), and heart (1 of 10). The modified Steiner stain was more sensitive in the detection of bacteria than PCR on FFPE samples. Fifteen kittens had comorbidities. A weakened immune state caused by maternal, environmental, infectious, and/or nutritional causes is speculated to have contributed to disease onset. We found that Tyzzer disease is more common than previously believed in orphaned kittens and should be considered in kittens with colitis and/or hepatitis.

Keywords: cats; *Clostridium piliforme*; colitis; hepatitis; Tyzzer disease.

Tyzzer disease is caused by *Clostridium piliforme*, a gram-negative, spore-forming, rod-shaped, piliform, flagellated, obligate intracellular bacterium, which in some animal species is associated with a classical triad of lesions, namely colitis, hepatitis, and myocarditis.[9] The disease is most often reported in rodents and small mammals including rabbits,[9] as well as horses,[3] among domestic species. In other domestic species, sporadic reports of Tyzzer disease exist in kittens,[1,5,7,10–12] puppies,[6,18] calves,[4] and a lamb.[13] However, Tyzzer disease is not considered to be significantly prevalent in these species.

In 2021, increased autopsy submissions of orphaned kittens at the Veterinary Medical Teaching Hospital of the University of California–Davis (UCD-VMTH) led to the identification of multiple cases of Tyzzer disease. Seven of 47 (14.9%) kittens, 0–35-d-old, autopsied in 2021, were confirmed to have Tyzzer disease based on intraepithelial silver stain–positive rods in the colon and/or liver. Given this high prevalence, we hypothesized that *C. piliforme* is an important infectious agent in kittens. Therefore, we performed a retrospective study to 1) identify the prevalence of Tyzzer disease in kittens with colitis and/or hepatitis with or without myocarditis using a modified Steiner stain, and/or a PCR assay on formalin-fixed, paraffin-embedded (FFPE) samples, and 2) characterize the gross and histologic lesions of Tyzzer disease in kittens.

The database of the pathology archives of UCD-VMTH, was searched for the keywords “colitis”, “hepatitis”, and “myocarditis” restricting the search to kittens <6-mo-old that had received an autopsy between January 1, 2000, and December 31, 2021; 37 cases were identified. Archived H&E-stained sections and sections of colon, liver, and heart from 36 of these cases stained with a modified Steiner stain were examined by a pathology resident in training (S. Fingerhood) and a board-certified pathologist (E.A. Choi). Slides or blocks from one case were not available, but the case was included in our study based on the pathology report.

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Intracellular filamentous bacteria consistent with *C. piliforme* were identified in 19 of 37 cases, consistent with infection by this microorganism. Of these 19 cases, 10 were selected for PCR analysis of FFPE sections of colon, liver, and heart, following a described protocol.[3] PCR results were reported as negative, suspected, or positive.

Briefly, the median age of affected kittens was 17.5 d (range: 7–42 d) and the median weight was 200 g (range: 128–470 g; Table 1, Suppl. Table 1). Eleven were female and 8 were male. Most were domestic shorthaired cats, except for 1 domestic medium-haired cat and 1 Siamese. Five kittens were enrolled in a study evaluating a probiotic, but they had not yet begun the trial (UC Davis IACUC protocol 2102). All kittens except case 7 were orphaned kittens being fostered with unknown history prior to being found. Three sets of siblings were included in our study (cases 9 & 10, 11 & 12, 14 & 15). Additional littermates died (case 1), or also had diarrhea but survived (cases 13, 16). Diarrhea was reported in 14 of 19 kittens. Fifteen kittens had comorbidities including sepsis (10 of 15), coccidiosis (4 of 15), feline parvovirus (FPV; *Carnivore protoparvovirus 1*) infection (2 of 15), upper respiratory tract infection (2 of 15), aspiration pneumonia (1 of 15), and intestinal foreign body (1 of 15). *C. piliforme* infection was considered the sole cause of death in kittens 3, 13, 16, and 19. Aerobic culture of liver was performed in 4 cases: cultured hemolytic *Escherichia coli* (case 4), *Streptococcus canis* (cases 8, 9), and mixed bacteria of unknown significance (case 10).

Consistent gross findings at the time of autopsy were poor nutritional condition, based on minimal subcutaneous and visceral adipose stores. Lymphoid depletion of the spleen, thymus, and/or lymph nodes was mentioned in 10 of 19 reports. Gross descriptions of the large intestine were available in 13 of 19 cases and mostly concerned the luminal content, which was described to be pasty and yellow-white (3 of 13), white mucoid (3 of 13), gray-brown (3 of 13), green-yellow (2 of 13), or gray-white (2 of 13). The intestinal walls were described as abnormal: thick and ropey in case 3, and broad coalescing discolored patches associated with non–*C. piliforme*-associated sepsis in case 17.

Gross liver lesions were appreciated in 5 cases and ranged from rare, randomly distributed, pinpoint, barely discernible pale-tan foci (cases 10, 16), tens of these foci (case 8), to florid, densely packed, pale-tan foci that occasionally had a red target-like appearance (cases 9, 13; Fig. 1).

Histologically, mild-to-severe, ulcerative and/or erosive colitis, typhlitis, or typhlocolitis was present in all but 1 kitten (18 of 19), which did not have the large intestine examined histologically. Ileitis was present in 7 of 19 cases for which sections of the ileocecal-colic junction were available. Proctitis was noted in kitten 18. In affected segments, the most common findings were neutrophilic crypt abscesses, variable crypt degeneration and milder regeneration, and/or crypt loss along with a variably mixed inflammatory infiltrate (Fig. 2). A lymphohistiocytic and variably neutrophilic infiltrate was often present, with the depth of the inflammation extending to the muscularis in severely ulcerated cases. Intraepithelial rods were difficult to identify in most H&E-stained sections. Hepatitis with hepatocellular necrosis and variable numbers of neutrophils was present in 11 of 19 cases (Suppl. Fig. 1); the lesion was not obvious grossly in 6 of these cases. The distribution of the hepatic necrosis was random and mostly infrequent-to-occasional, and in some cases, very rare and did not affect all lobes (e.g., kitten 16 had 3 hepatic necroinflammatory foci in 3 of 7 sections of liver examined). In these 11 cases, hepatocytes at the edge of the necrotic foci had intracytoplasmic pale basophilic linear stacks of rods, supporting *C. piliforme* infection (Suppl. Fig. 2). Myocarditis was noted in kitten 9, which also had streptococcal sepsis characterized by small colonies of cocci in foci of myocarditis. No intracytoplasmic rods were observed in the heart of this cat, and the cause of myocarditis was considered to be *Streptococcus* sp. and not *C. piliforme*. Of the 19 cases, cases 3, 8, 13, and 18 had perianal dermatitis observed grossly, and 2 of these 4 cases (cases 3, 18) had perianal skin reviewed histologically. The main lesion was ulcerative dermatitis with mixed bacteria (Fig. 3).

The modified Steiner stain on affected colon and liver sections revealed intracellular stacks of slender argyrophilic bacteria, consistent with *C. piliforme*, within colonic enterocytes in 18 of 19 cases and within hepatocytes in 11 of 19 cases (Table 1). In all cases, the intracellular bacteria were associated with necroinflammatory foci in the colon and liver. In the Steiner-stained liver, stacks of intraepithelial bacteria were found along the periphery of the necrotic regions as expected, and individual extracellular rods were identified throughout the necroinflammatory foci (Suppl. Figs. 3, 4). The modified Steiner revealed intracardiomyocytic rods in case 13, with no evidence of myocarditis in the H&E-stained section. In the most severely affected kitten with perianal dermatitis (case 3), the Steiner stain highlighted stacks of bacteria within follicular keratinocytes spilling into the follicular lumen where the follicular epithelium was necrotic and/or absent (Fig. 4). In kitten 18, with much milder perianal dermatitis, the Steiner stain highlighted rare intrakeratinocytic rods.

A PCR assay performed on FFPE samples confirmed *C. piliforme* infection in 5 of 10 cases and was suspected in 2 additional cases. Most positive or suspected cases were from the colon and liver (Table 1), as expected from the silver stain. In case 13, rods identified in the Steiner stain were correlated with suspect heart PCR positivity.

The predominant histologic lesion associated with Tyzzer disease in these 19 kittens was colitis. This agrees with the clinical history of diarrhea seen in these kittens. The utility of a silver impregnation (i.e., modified Steiner) stain in the diagnosis of Tyzzer disease is also highlighted by our results, given that bacteria were not initially identified in H&E-stained sections of most archived cases. Although gross and histologic evidence of colitis and hepatitis are suggestive of Tyzzer

Table 1. Summary of signalment and lesions found in 19 kittens with Tyzzer disease.

				Colon				Liver			
Case	Age, d	Wt, g	Sex	Gross description	Histologic colitis	Mod. Steiner	PCR	Gross hepatitis	Histologic hepatitis	Mod. Steiner	PCR
1	42	300	F	NA	+	+	–	–	–	–	–
2	30	300	M	NA	+	+	–	–	+	–	–
3	7	147	F	Thickened wall, yellow-white contents	+	+	+	–	+	+	+
4	28	200	F	White mucoid contents	+	+	NA	–	–	–	NA
5	22	222	F	NA	+	+	–	–	+	+	–
6	NA	250	F	Green-yellow contents	+	+	NA	–	–	–	NA
7	42	470	F	Gray-brown contents	+	+	NA	–	–	–	NA
8	14	200	M	NA	+	S	+	+	+	+	–
9*	14	179	F	Gray-white contents	+	+	–	+	+	+	+
10*	10	176	F	Gray-white contents	+	+	–	+	+	+	S
11†	12	165	M	NA	+	+	S	–	+	+	S
12†	14	180	M	Yellow-white contents	+	+	+	–	–	–	–
13§	23	300	M	Gray-brown contents	+	+	S	+	+	+	+
14‡	10	128	M	White mucoid contents	+	+	NA	–	–	+	NA
15‡	10	150	M	Gray-brown contents	+	+	NA	–	–	–	NA
16	21	200	F	Yellow-white contents	+	NA	NA	+	+	+	NA
17	28	160	M	Mural discolored patches with white mucoid contents	+	+	NA	–	–	–	NA
18	28	300	F	Green-yellow contents	+	+	NA	–	+	+	NA
19	14	NA	F	NA	+	+	NA	–	+	+	NA

F = female; M = male; Mod. Steiner = modified Steiner stain; NA = not available; S = suspected.
*, †, ‡ Siblings.
§ Formalin-fixed, paraffin-embedded heart PCR suspected and intracellular bacteria confirmed with modified Steiner.

disease, other infectious agents are also capable of producing indistinguishable lesions, highlighting the importance of performing silver staining to identify intracellular bacteria.

Although the histologic lesions observed are considered pathognomonic for Tyzzer disease, other supportive tests include PCR on fresh or FFPE samples, serology, immunohistochemistry, or electron microscopy.[3,10] We chose to perform a PCR assay on FFPE samples on a subset of cases, especially given the usefulness of PCR reported in a study in foals[3]; however, in our study, PCR was not as sensitive as modified Steiner staining. We speculate that the greater sensitivity of Steiner staining may result from 1) the smaller bacterial burden in kittens, given that most cases in horses were described to have florid evidence of bacterial load, and 2) the inherent issues with performing PCR on FFPE tissues, particularly related to the effects of fixation on DNA fragmentation but also in the formation of cross-linked proteins that make tissue digestion, and therefore DNA extraction, more challenging.[15] The young age distribution of our identified cases is consistent with that described in other species. Interestingly, our series of cases included 3 sets of siblings, which could reflect similar environmental conditions (e.g., stress, high environmental load), pathogenic strains of the bacteria, and/or genetic susceptibility, as has been described in certain strains of mice.[16]

C. piliforme has historically not been considered to be a major pathogen associated with diarrhea and sepsis in kittens, as it is in rodents, small mammals, and foals. To date, 7 case reports and case studies have described Tyzzer disease in cats, accounting for a total of 13 published cases.[1,5,7,8,10–12] The age of diagnosis ranged from 4-wk-old to 10-y-old[12] but most cases are 4–8-wk-old or the peri-weaning period. This contrasts with our study in which most cases were in 1–6-wk-old kittens. However, similarly, most previously reported kittens had coinfections, including infection by feline leukemia virus (FeLV; experimental),[1] FPV,[5] feline infectious peritonitis virus (*Alphacoronavirus 1*),[5,8] and felid herpesvirus 1 (*Felid alphaherpesvirus 1*).[10] These coinfections were considered to have caused immunosuppression and contributed to *C. piliforme* infection. A few studies noted that thymic atrophy was a consistent gross finding, and speculated that environmental factors led to stress-induced immunosuppression,[1,5,10] as was seen in 10 of 19 of our cases.

Other potential causes of increased susceptibility include nutritional deficiencies or excess, as reported in foals and fawns,[2,3] failure of passive transfer, and additional infectious systemic or intestinal comorbidities. Most of the kittens in our study were known to have been born to feral queens, and exposure to the bacteria is presumed to have been environmental. Feral cats, which tend to hunt rodents, may be more

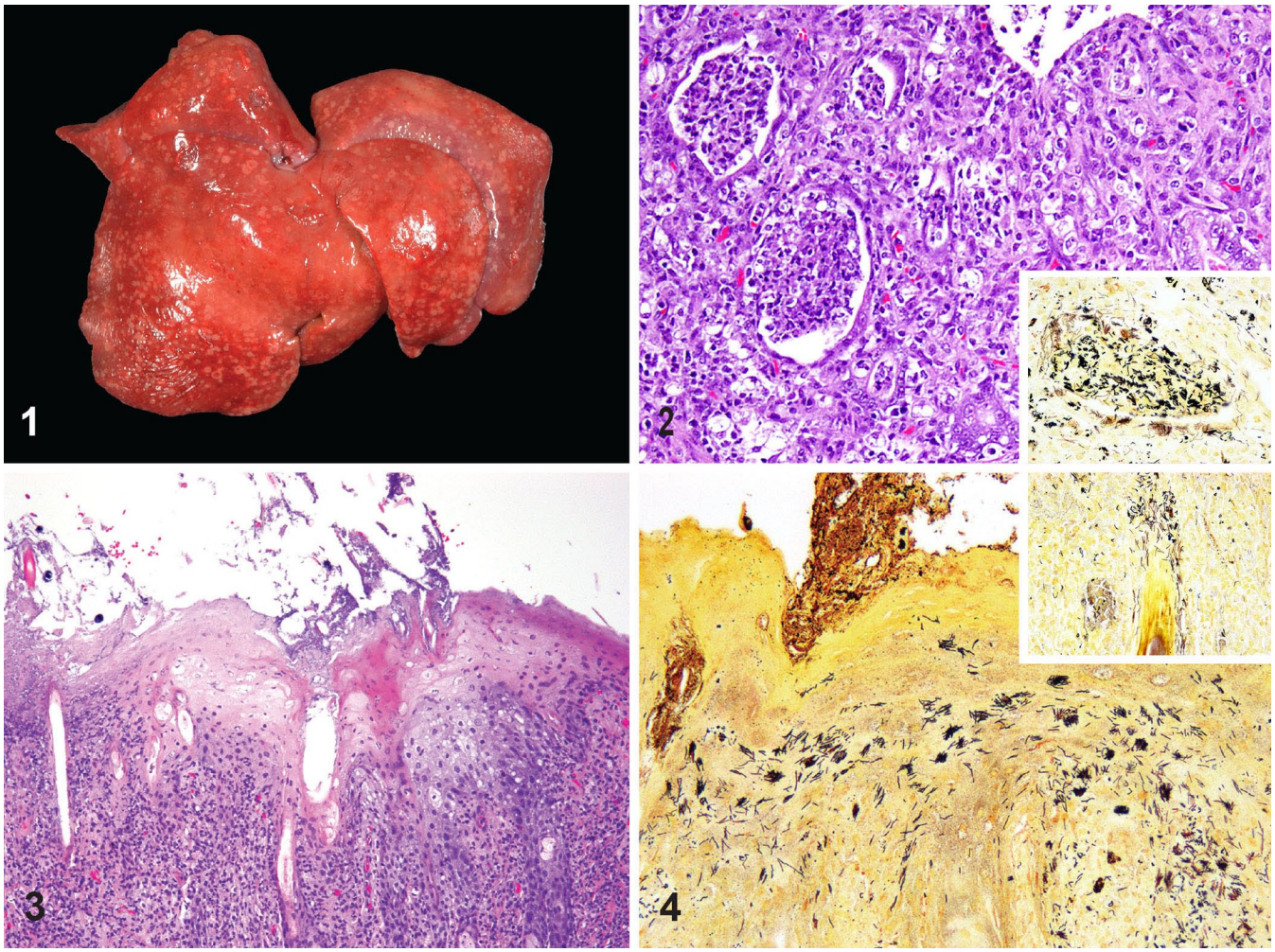

Figure 1. Liver of kitten 13 with Tyzzer disease. Frequent pale foci are scattered throughout the liver. The corresponding histology is presented in Suppl. Figs. 1–4. **Figures 2–4.** Microscopic lesions in kitten 3 with Tyzzer disease. **Figure 2.** Colitis characterized by ulceration and neutrophilic crypt abscesses, with lamina propria histiocytic and lymphocytic infiltrate. H&E. Inset: stacks of brown intraepithelial rods in attenuated crypt epithelial cells. Modified Steiner stain. **Figure 3.** Perianal ulcerative dermatitis. The epidermis is edematous, and the dermis is severely infiltrated by mixed leukocytes. H&E. **Figure 4.** Stacks of intraepithelial bacteria within epidermal keratinocytes and follicular epithelium. Inset: higher magnification. Modified Steiner stain.

exposed than other domestic species. Given how often coinfections were identified in kittens with Tyzzer disease, we believe that exposure to contaminated soil and immunosuppression caused by environmental, immunologic or infectious, and nutritional factors, may be significant predisposing factors for this disease. Based on our study and literature review, it appears that preweaned to weaned kittens historically or recently exposed to *C. piliforme* may develop the disease if their immune status is inadequate (orphaned kitten) or when the immune system is compromised (e.g., kitten with other diseases and/or stress) by activation of latent infections after exposure.[17] The latter is supported by a study that reported Tyzzer disease in FeLV-infected kittens kept in a closed experimental environment for 7 wk before being found dead of Tyzzer disease.[1] Succumbing to the disease appears to be rare in adult cats, but they may develop diarrhea.[12]

Histologically, FPV infection was considered a major differential diagnosis in many affected kittens given crypt dilation and crypt epithelial damage; however, compared to FPV infection, the neutrophilic crypt abscesses and general inflammatory infiltrate seemed more robust, and the crypt epithelial regenerative effort was less dramatic. The variability of neutrophils involved was speculated to be associated with the presence of erosion or ulceration and the degree of the innate immune response.[14]

Interestingly, cases 3, 8, 13, and 18 had perianal dermatitis; 2 of 4 had perianal skin reviewed histologically. The main lesion was ulcerative dermatitis with mixed bacteria (Fig. 3) and, in both cases, intraepithelial stacks of rods were identified in the epidermis and follicular epithelium compatible with *C. piliforme* involvement in the skin (Fig. 4). *C. piliforme*–associated dermatitis has only been described in

an immunocompromised man, seen as a verrucous hyperplastic nodular dermatitis on the chest.[14] Our cases were ulcerative and likely more acute. We believe this to be an opportunistic infection further to perianal fecal staining, suggesting that local infection can occur with immune barrier breakdown.

Our results suggest that *C. piliforme* infection is a major contributing cause of death in orphaned kittens, especially in 2–4-wk-olds, and mainly causes diarrhea associated with ulcerative colitis and/or typhlitis and ileitis, less often hepatitis, and rarely proctitis and perianal ulcerative dermatitis. Histologically, FPV infection is a major differential consideration,[7] and diagnostically both agents should be considered given that coinfections occur. As in foals, myocarditis appears to occur very rarely. Studies are warranted to determine whether timely management of infected kittens with antimicrobials such as tetracycline, streptomycin, erythromycin, or penicillin could reduce morbidity and mortality. Tyzzer disease should be a differential diagnosis for the cause of death in kittens, especially those with poor nutritional condition and/or immunosuppression.

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Supplemental material

Supplemental material for this article is available online.

References

1. Bennett AM, et al. Tyzzer's disease in cats experimentally infected with feline leukaemia virus. Vet Microbiol 1977;2:49–56.
2. Brooks JW, et al. *Clostridium piliforme* infection in two farm-raised white-tailed deer fawns (*Odocoileus virginianus*) and association with copper toxicosis. Vet Pathol 2006;43:765–768.
3. García JA, et al. *Clostridium piliforme* infection (Tyzzer disease) in horses: retrospective study of 25 cases and literature review. J Vet Diagn Invest 2022;34:421–428.
4. Ikegami T, et al. Naturally occurring Tyzzer's disease in a calf. Vet Pathol 1999;36:253–255.
5. Ikegami T, et al. Enterocolitis associated with dual infection by *Clostridium piliforme* and feline panleukopenia virus in three kittens. Vet Pathol 1999;36:613–615.
6. Jacobson SA, et al. *Clostridium piliforme* and canine distemper virus coinfection in 2 domestic dog littermates and a gray fox kit. J Vet Diagn Invest 2022;34:894–897.
7. Kovatch RM, Zebarth G. Naturally occurring Tyzzer's disease in a cat. J Am Vet Med Assoc 1973;162:136–138.
8. Kubokawa K, et al. Two cases of feline Tyzzer's disease. Jpn J Exp Med 1973;43:413–421.
9. Navarro MA, Uzal FA. Pathobiology and diagnosis of clostridial hepatitis in animals. J Vet Diagn Invest 2020;32:192–202.
10. Neto RT, et al. Coinfection with *Clostridium piliforme* and Felid herpesvirus 1 in a kitten. J Vet Diagn Invest 2015;27:547–551.
11. Nimmo Wilkie JS, Barker IK. Colitis due to *Bacillus piliformis* in two kittens. Vet Pathol 1985;22:649–652.
12. Schneck G. Tyzzer's disease in an adult cat. Vet Med Small Anim Clin 1975;70:155–156.
13. Scholes SFE, Edwards GT. Tyzzer's disease (*Clostridium piliforme* infection) and possible copper toxicity in a lamb. Vet Rec 2009;164:470–471.
14. Smith KJ, et al. *Bacillus piliformis* infection (Tyzzer's disease) in a patient infected with HIV-1: confirmation with 16S ribosomal RNA sequence analysis. J Am Acad Dermatol 1996;34:343–348.
15. Srinivasan M, et al. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 2002;161:1961–1971.
16. Van Andel RA, et al. Interleukin-12 has a role in mediating resistance of murine strains to Tyzzer's disease. Infect Immun 1998;66:4942–4946.
17. Wobeser G, et al. Tularemia, plague, yersiniosis, and Tyzzer's disease in wild rodents and lagomorphs in Canada: a review. Can Vet J 2009;50:1251–1256.
18. Young JK, et al. Naturally occurring Tyzzer's disease in a puppy. Vet Pathol 1995;32:63–65.